REMARKS

Status of Claims

Claims 61-97 are currently pending. Claims 1-60 have been canceled without prejudice or disclaimer of the subject matter claimed therein. New claims 61-97 directed to the same invention as claims 1-60 have been added.

Amendments to the Specification

The specification has been amended to replace the term "anthrax" which refers to a disease with the bacterium "bacillus anthracis" which causes the disease, since the paragraph discloses various bacteria that cause diseases. Since "bacillus anthracis" is known as the bacterium causing anthrax, the amendment does not introduce prohibited new matter.

Amendments to the Claims

Claims 1-60 have been canceled and replaced with new claims 61-97. Representative support for new claims 61-97 is summarized in the table below. As shown in the table below, new claims 61-97 are essentially based on claims 1-60. New claims 61-97 do not include prohibited new matter.

Claims	Representative Support
61	Claim 2
62	Claim 5
63	Page 17, lines 14-16, 20; Page 14, line 38
64	Page 12, lines 6 and 7; Page 17, lines 16 and 17
65	Claim 11
66	Page 12, lines 6 and 7
67	Claims 47 and 48
68	Page 12, lines 4-8
69	Page 12, lines 4-8; Page 17, lines 14-19; Page 42, Example 4
70	Claim 11
71	Claim 46

72	Page 19, line 17
73	Claim 48
74	Claim 16
75	Claim 7
76	Claim 6
77	Claim 49
78	Claim 19
79	Claim 50
80	Claim 61
81	Claim 31; Page 37, line 30
82	Claim 35
83	Claim 31
84	Claim 3
85	Claim 51
86	Claim 51
87	Claim 4
88	Page 26, lines 24 to Page 27, line 2
89	Page 26, line 30
90	Page 26, lines 30-33
91-93	Page 26, lines 5-10
94	Page 26, lines 20 and 21
95	Page 26, lines 24 to Page 27, line 2
96	Page 26, line 30
97	Page 26, lines 30-33

Claim Rejections under 35 U.S.C. § 112, First Paragraph

A. Claims 2-7, 11, 13-14, 16, 19, 31, 38 and 46-60 are rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the enablement requirement.

The Office Action alleges that the specification while being enabling for a method of inducing an immune response by applying a formulation onto pretreated skin, does not

reasonably provide enablement for a method of inducing an immune response by applying a formulation onto skin that is dry or intact. The Office Action also cites the factors set forth in *In re Wands* in support of their position.

Claims 1-60 have been canceled without prejudice or disclaimer of the subject matter claimed therein and replaced with new claims 61-97. Claim 61 and its dependent claims 62-97 are directed to a method of inducing an immune response comprising applying a dry formulation to the skin of a subject, wherein the formulation comprises an antigen and an adjuvant. Applicants respectfully point out that the claims do not require that the level of immune response induced by the dry formulation be the same or higher than the immune response induced with pretreatment of the skin. Moreover, as shown in Example 2, specifically, page 40, lines 20 and 21, the claimed invention includes a method of inducing an immune response comprising delivery of a dry formulation by transcutaneous immunization with and without pretreatment of the skin.

In the response submitted on October 7, 2005, Applicants selected representative examples from the specification as evidence showing induction of antibodies upon application of dry formulation to skin without pretreatment for showing enablement of the scope of the claims by the specification. Applicants reiterate the representative examples in this response.

As pointed out in the last response, the specification demonstrates in Example 1 that a dry powder formulation of Cholera toxin (CT) induced high levels of antibodies in mice when applied to skin (see, *e.g.*, page 38, lines 3 to 8). The data in Table 1 confirm that mice immunized on the skin with CT in the form of dry powder achieved high titers of antibody without pretreatment. Mice numbers 996, 997, 998, 999 were immunized on the skin with CT in the form of dry powder without pretreatment, and anti-CT antibodies in these mice were detected by ELISA two weeks after initial immunization. As shown in Table 1, the geometric mean of the 14-day titer for these mice is 4957 (ELISA Units), while their prebleed is < 5. Applicants respectfully point out that as indicated on page 38, lines 1 and 2 of the specification, the geometric mean is calculated from the subtracted titers (14-day titer minus prebleed).

Additionally, the data in Tables 2 and 3 summarize similar results for Examples 2 and 3, respectively, using immunizations with reduced amounts of CT (50 μg and 25 μg respectively) in powder form applied to the skin. In Table 2, mice numbers 11712 to 11716 and in Table 3, mice numbers 836 to 840 were immunized in a similar manner as in Table 1. Likewise, as

shown in both of these tables, the mice developed antibodies against CT when CT was applied in dry form to the skin of the mice without pretreatment.

In addition, Examples 4 through 7 disclose data showing that dry formulations of different antigens induce an immune response when placed on the skin. In each Example, a specified amount of an antigen solubilized in a liquid solution was allowed to dry overnight to achieve a dry formulation. Then the dry formulation was placed on the skin of mice to test for an induction of an immune response. The data collected show an immune response to each antigen when a dry formulation of each antigen was placed on the skin of mice (Tables 4-11). The amount of antibodies induced by the dry formulation in each case is higher than that in the prebleed.

Although the amount of antibodies induced in Tables 1-11 may not be as high as the amount of antibodies induced after pretreatment of the skin, the dry formulation applied without pretreatment induced an immune response in these mice, since the 14-day titer had a higher level of anti-CT antibodies than the prebleed. As pointed out above, the claims do not require that the level of antibodies induced by the dry formulation applied to the skin be the same as or greater than that induced with pretreatment. The claims only require an induction of an immune response by the dry formulation. The data in Tables 1-11 show that applying a dry formulation to the skin without pretreatment induces an immune response.

Moreover, the attached §1.132 declaration by Dr. Diane Epperson shows that a dry formulation containing antigen and adjuvant applied to the skin of a subject without pretreating the skin induces an immune response. Briefly, a dry formulation comprising influenza split virus and hemagglutinin applied to the skin of guinea pig induced an increase in antibody titers against the influenza strain (see graphs in declaration). The skin of the guinea pig was not hydrated or pretreated in any manner prior to application of the dry formulation.

The Advisory Action, dated June 5, 2006, alleges that the specification includes pretreatment of the skin chemically or physically to enhance penetration. However, as stated in the specification, on page 7, lines 30 and 31, the invention "may be practiced with chemical or physical enhancement" and on page 40, lines 20 and 21, the invention may be practiced "with or without pretreatment of the skin." Thus, the invention need not be practiced with pretreatment.

The Advisory Action also alleges that shaving the skin of the animal is a form of pretreatment. Applicants respectfully point out that the animals were shaved to remove their fur,

so that their skin would more closely resemble that of a human subject. Moreover, as described in the specification, on page 32, lines 17-20, the shaving was performed without signs of trauma to the skin and the shaving was performed 48 hours prior to immunization to allow the animals to rest and their skin cells to return to their normal state. Thus, the shaving to remove the fur of the animals would not have any affect on the penetration of the formulation.

Nevertheless, Applicants submit a §1.132 declaration by Dr. Gregory Glenn to show that pretreatment is not necessary to induce an immune response. The attached §1.132 declaration by Dr. Gregory Glenn confirms that dry formulation delivered to human patients by transcutaneous immunization without pretreatment or manipulation of their skin in any manner (such as shaving) was able to induce an immune response in the patient. The declaration confirms that shaving is not required to induce an immune response. Thus, the specification enables a method of inducing an immune response comprising administering a dry formulation by transcutaneous immunization without pretreatment of the skin.

In summary, these data demonstrate that a dry formulation applied directly to the skin without pretreatment induces an immune response. The Examples describe in detail how the immunizations were accomplished thus teaching one skilled in the art how to use the claimed invention. The attached declarations provide further support of the teachings of the specification. Therefore, the specification provides sufficient guidance to enable one of ordinary skill in the art to make and use a dry formulation comprising an antigen and an adjuvant for inducing an immune response without undue experimentation. Applicants respectfully point out that the Examples in the specification describe in detail how the dry formulations were made, how they were applied, and how to test for induction of an immune response. Accordingly, the specification has satisfied the requirements of enablement set forth by the Wands factors. Applicants respectfully request that this rejection be withdrawn.

Previous Rejections under 35 U.S.C. § 102 and § 103 (Office Action dated July 8, 2005)

A. In the previous Office Action (dated July 8, 2005), claims 2-5, 7, 11, 13, 14, 16, 19, 31, 38, 46-48, 51-54, 56, and 58-60 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent 5,910,306 and claims 2-7, 11, 13, 14, 16, 19, 31, 38, and 46-60 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent 5,980,898.

Claims 1-60 have been canceled without prejudice or disclaimer of the subject matter claimed therein and replaced with new claims 61-97. Claim 61 and its dependent claims 62-97 are directed to a method of inducing an immune response comprising applying a dry formulation to the skin of a subject, wherein the formulation comprises an antigen and an adjuvant. The cited patent does not anticipate claims 61-97.

The previous Office Action alleges that claims 1-60 are anticipated by the cited patents because the cited patents teach that liposomes containing antigen and adjuvant can be lyophilized and disclose application of the formulation to dry skin of subject. However, Applicants respectfully point out that the cited patents do not teach applying dry formulations comprising liposomes to the skin. Rather, the cited patents disclose that the formulations were prepared by mixing lyophilized liposomes with antigen that were dissolved or suspended in solution. Accordingly, the formulations comprising liposomes and antigen disclosed by the patents were wet solutions not dry formulations (see U.S. Patent 5,910,306, col. 4, lines 33-35 and col. 12, Example 2; and U.S. Patent 5,980,898 col. 11, line 63 to 65 and col. 12, lines 36-39).

Moreover, the Office Action cites col. 12, lines 44-46 of U.S. Patent 5,980,898 as providing support for dry formulation. Applicants respectfully point out that lines 44-46 of the cited patent by stating, "although not required to practice the present invention, hydration and/or penetration of the stratum corneum may be enhanced by adding liposomes to the formulations," only suggests that liposomes may be added to hydrate the skin. This statement does not teach or show applying dry formulation containing liposomes to the skin of the subject.

Thus, the cited patents do not anticipate the claimed invention.

B. In the previous Office Action (dated July 8, 2005), claims 2, 6, 49, 55 and 57 were rejected under 35 U.S.C. § 103 as being unpatentable over U.S. Patent 5,910,306 in view U.S. Patent 5,988,898.

As discussed above, claims 1-60 have been canceled and replaced with new claims 61-97. Applicants respectfully submits that the cited patents do not render claims 61-97 obvious.

The deficiencies of the cited patents are discussed immediately above. The cited references do not disclose applying dry formulations comprising an antigen to the skin of a subject to induce an immune response. The formulations disclosed in the cited patents are solutions comprising antigen and liposomes. The cited patents do not provide evidence that a

dry formulation would induce an immune response. Thus, the cited patents do not provide motivation to modify their teachings and to obtain a method of inducing an immune response by applying dry formulations.

Accordingly, the cited patents do not render the claimed invention obvious.

Rejections under 35 U.S.C. § 102

A. Claims 2-7, 11, 13, 14, 16, 19, 31, 38, 46-60 were rejected under 35 U.S.C. § 102 (e) as being anticipated by U.S. Patent 6,797,276.

Claims 1-60 have been canceled and replaced by claims 61-97. Applicants respectfully submit that the cited patent does not anticipate claims 61-97. As discussed above, claim 61 and its dependent claims 62-97 are directed to a method of inducing an immune response comprising applying a dry formulation to the skin of a subject, wherein the formulation comprises an antigen and an adjuvant.

The Office Action alleges that the cited patent teaches a method of inducing immune response comprising administering a dry formulation because the cited patent discloses that the antigenic formulation can be utilized with vehicles encompassing powder.

Applicants respectfully point out that the cited patent does not teach dry formulation for inducing an immune response. The cited patent discloses pretreatment of the skin prior to administering a formulation comprising an antigen. The paragraph (col. 8, line 25 of U.S. Patent 6,797,276) that the Office Action cited to teaches that the skin may be hydrated or made permeable by using various vehicles including powder. The paragraph does not teach or disclose applying dry formulations comprising antigen to the skin of a subject. As shown in the Examples of U.S. Patent 6,797,276, the formulations that were applied to the skin of the subject were wet formulations, *i.e.* solutions.

The Office Action alleges that claims 1-11 and col. 8, line 25 of the cited patent discloses a method of inducing an immune response with a dry formulation. Applicants respectfully submit neither the claims nor the specification of the cited patent discloses applying dry formulation to induce an immune response. Col. 8, line 25, as mentioned above, discusses the various "vehicles" that may be used to hydrate the skin which is pretreatment of the skin. The cited patent teaches that the "vehicles" that may be used to treat the skin includes humectants, powders, emulsions, and occlusive dressing. The cited patent does not teach or disclose that the

formulations used with these "vehicles" must be dry formulations, and the cited patent does not disclose a method of inducing an immune response comprising applying a dry formulation to the skin of a subject. Thus, even though the cited patent discloses pretreating the skin with alcohol, the cited patent does not teach applying dry formulation to pretreated skin.

In contrast, the claims as they stand are directed to a method of inducing an immune response by applying a dry formulation to the skin of a subject. Accordingly, the cited patent does not anticipate the claimed invention.

Non-Statutory Double Patenting

Claims 2-5, 11, 13, 14, 31, 38, 46-48, 50-54, 57, and 58 were rejected under the ground of nonstatutory obviousness-type double patenting. Specifically, the claims were rejected over claims 1-11 of U.S. Patent 6,797,276.

Claims 1-60 have been canceled and replaced with claims 61-97. Respectfully, Applicants would like to point out that claims 61-97 are directed to a method of inducing an immune response comprising applying a dry formulation to skin of a subject. Although the claims of U.S. Patent 6,797,276 are directed to a method of inducing an immune response, the claims of the cited patent are not directed to a method of inducing an immune response comprising applying dry formulation to the skin of a subject. Moreover, the cited patent does not disclose dry formulations for inducing an immune response. As discussed above, the formulations disclosed by the cited patent are solutions. Accordingly, the claims of the present application and the claims of the cited patent are directed to different inventions. Therefore, Applicants assert that the claims in the present application are patently distinct from the pending applications.

CONCLUSION

The foregoing amendments and remarks are being made to place the application in condition for allowance. Applicants respectfully request entry of the amendments, reconsideration, and the timely allowance of the pending claims. A favorable action is awaited. Should the Examiner find that an interview would be helpful to further prosecution of this application, they are invited to telephone the undersigned at their convenience.

If there are any additional fees due in connection with the filing of this response, please

charge the fees to our Deposit Account No. 50-0310. If a fee is required for an extension of time under 37 C.F.R. §1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

MORGAN, LEWIS & BOCKIUS LLP

Dated: October 10, 2006

By: _____
Sally P. Teng, Ph.D.
Reg. No. 45,397

CUSTOMER NO. 09629
MORGAN, LEWIS & BOCKIUS LLP
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004
Phone: (202)739-3000

Transcutaneous immunization: A human vaccine delivery strategy using a patch

GREGORY M. GLENN[1,2], DAVID N. TAYLOR[3], XIURU LI[2], SARAH FRANKEL[4], ANDREW MONTEMARANO[5] & CARL R. ALVING[1]

[1]*Department of Membrane Biochemistry, Walter Reed Army Institute of Research, 503 Robert Grant Rd., Rm. 2W-124, Silver Spring, Maryland 20910-7500, USA*
[2]*IOMAI Corporation, Washington, DC 20037, USA*
[3]*Department of Enteric Infections, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500, USA*
[4]*Armed Forces Institutes of Pathology, Washington, DC 20307, USA*
[5]*Department of Dermatology, Walter Reed Army Medical Center, Washington, DC 20307, USA*
Correspondence should be addressed to G.M.G.; email: gglenn@iomai.com

Transcutaneous immunization, a topical vaccine application, combines the advantages of needle-free delivery while targeting the immunologically rich milieu of the skin. In animal studies, this simple technique induces robust systemic and mucosal antibodies against vaccine antigens. Here, we demonstrate safe application of a patch containing heat-labile enterotoxin (LT, derived from *Escherichia coli*) to humans, resulting in robust LT-antibody responses. These findings indicate that TCI is feasible for human immunization, and suggest that TCI may enhance efficacy as well as improve vaccine delivery.

Transcutaneous immunization (TCI) is a new method of vaccination that utilizes a topical application of an adjuvant and vaccine antigen to intact skin to induce an immune response[1]. Bacterial products such as LT and cholera toxin (CT) are members of a class of potent molecules known as adjuvants used to enhance immune responses to vaccine components. CT and LT are used extensively by the oral and nasal routes to enhance the mucosal immune response to vaccines[2-4]. When applied to the skin of animals, both CT and LT induce systemic and mucosal immune responses to themselves and to co-administered antigens such as diphtheria and tetanus toxoids that are administered along with these adjuvants[5]. In animal studies, adjuvants such as CT and LT are essential for the induction of robust immune responses via the skin.

Needle-free delivery has become a global priority because of the risk of needle-borne diseases associated with re-use and improper disposal of needles[6]. Vaccine delivery in a patch has other obvious practical merits. Vaccination through the skin may be particularly advantageous, as the epidermis is replete with immune cells called Langerhans cells (LCs). LCs are in close proximity to the most superficial layer of the skin, the stratum corneum, and represent a network of immune cells that underlie 25% of the skin's total surface area[7]. The stratum corneum, composed of dead cells and lipids, represents the principal barrier to skin penetration. This barrier can be readily disrupted by hydration via simple occlusion of the skin with a patch [8], possibly allowing antigens into the epidermis. In contact sensitization, LCs carry antigen to the draining lymph nodes where systemic immune responses are induced[9]. The induction of immune responses by TCI suggests that LCs are similarly engaged.

CT and LT act as both adjuvants and antigens[2,10], inducing antibodies against CT and LT when applied to the skin[5]. Antibodies against toxins contribute to protection against human diarrheal disease[11] and, in mice, both serum and mucosal IgG and IgA antibodies against toxins can be detected in response to TCI. In the present study, we tested whether LT delivered transcutaneously is safe and immunogenic in humans.

TCI in humans

A graded-dose phase I trial was conducted in 18 volunteers to evaluate the safety and immunogenicity of LT delivered in a patch. Four volunteers received doses of either 25, 50 or 250 μg LT. Six volunteers received 500 μg LT. The LT solution was added to a gauze pad under an adhesive patch and applied to the upper arm for six hours. Volunteers were monitored for systemic or local reactions at one, two, three and seven days post-vaccination. All volunteers were boosted at 12 weeks. The group receiving 500 μg LT returned for a third immunization 35 weeks after the first immunization, and 250 μg LT was administered by patch to each arm.

No serious vaccine-related adverse reactions were observed,

Table 1 Individual and mean Anti-LT antibody responses at day 312[a]

Volunteer	Anti-LT IgG	Fold rise IgG	Anti-LT IgA	Fold rise IgA
1	10,271	17.6	4,213	6.9
2	5,098	4.7	1,426	4.4
3	13,468	16.8	2,563	5.6
4	12,317	10.0	3,632	5.1
5	18,301	27.3	11,607	13.2
6	8,238	11.3	6,268	12.3
Mean	10,460	14.6	3,996	7.2

[a], IgG reported as ELISA units and IgA reported as ng/mL. Data are from individuals immunized with 500 μg of LT.

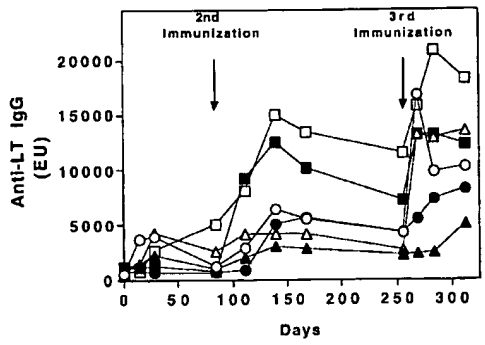

Fig. 1 Individual human serum IgG antibody responses to LT. Six volunteers were immunized using a patch containing 500 μg of LT placed on the upper arm for 6 h at 0, 12 and 35 wk. Individual IgG antibodies against LT are shown over the time course of the trial and reported as ELISA units (EU), the inverse dilution at which the sera yields an optical density of 1.0.

either systemically or at the site of immunization. One volunteer developed a mild dermatitis at the adhesive site. All subjects in the 500 μg group produced a greater than four-fold rise in serum IgG antibodies-against-LT with clear boosting responses (Fig. 1). There were minimal rises in other dosing groups. The antibodies against LT were durable and clearly persisted after the second immunization through 35 weeks (Fig. 1). After the third immunization, the geometric mean antibody titer in the 500-μg group was 10,460 ELISA units (EU), a 14.6-fold (mean) rise in antibody titer to LT (Table 1). Similarly, the same volunteers responded with a geometric mean of 3,996 ng/ml of IgA antibody against LT, a 7.2-fold (mean) rise in serum antibody titer (Table 1).

All individuals in the 500-μg group had detectable IgG or IgA antibody against LT in either the urine or stool. The individual antibody-against-LT titration curves for stool and urine antibodies for a single time point are illustrated in Fig. 2. As urine and stool samples vary in the total amount of immunoglobin present, the total IgG and IgA for each sample was determined and the sample's specific IgG and IgA were normalized for total IgG or IgA. Analysis of all the time points indicated that 3/6 and 2/6 subjects had a four-fold rise in urine IgA or IgG, respectively, and 3/6 and 3/6 subjects had a four-fold rise in IgA or IgG, respectively, in the stool. There was no clear correlation between the magnitude of the individual serum responses and antibodies in the urine or stool.

Microscopic findings in human skin biopsies

A biopsy was taken in two subjects at the site of immunization and the unimmunized arm at 24 and 48 hours after the second immunization, and from a third volunteer 48 hours after the third immunization from both an immunization site as well as a sham patch site. Hematoxylin and eosin staining of the specimens confirmed the clinical finding that no inflammation was seen after the immunization (Fig. 3*a*). Although routine histologic sections were unremarkable, LCs visualized using anti-CD1a staining consistently demonstrated enlarged or rounded LC cell bodies at the site of immunization at 24 and 48 hours (Fig. 3*b*, 48 h) compared to the control biopsies from the opposite arm (Fig. 3*c*, 48 h). LC morphology was unremarkable in the specimen from the sham patch, indicating that the observations were not related to the patch application itself. Changes in murine LC morphology after activation by bacterial products and cytokines are well described[12]. The LCs seen in human skin used for patch immunization were similar in appearance to tonsilar crypt LCs which are thought to be chronically activated by bacterial products from the flora of the mouth[13].

Discussion

We previously reported that TCI utilizing adjuvants and vaccine antigens could be used to effectively immunize animals[5,14]. The present study indicates that TCI safely induces systemic antibody responses in humans. The immunity induced by TCI appears to be durable, as indicated by the persistence of serum antibodies, and similar in magnitude to serum antibodies induced by oral immunization (12-fold rise) with LT in humans[3].

TCI in animals results in high levels of serum IgG and IgA antibodies, secondary antibody responses, $CD4^+$ T-cell responses, cytotoxic T cells (CTLs), and immune responses that are functional in challenge models[5,14-16]. In some settings, topical application has been shown to induce IgE[17]. In addition to systemic responses, IgG and IgA antibodies induced by TCI are readily detected at the mucosa[5,14]. Mucosal immunity encompasses a diverse set of local and distal elements including both IgG and IgA, and there is clearly communication between mucosal compartments[18]. The mechanism underlying the generation of antibodies detectable at the mucosa after TCI is yet to be determined. The finding, however, that IgG and IgA antibodies were detected in the urine and stool of volunteers using standard but insensitive detection tools indicates that human mucosal responses follow TCI. This finding is also consistent with studies on mice[5,14]. The induction of mucosal and systemic antibodies by TCI against pathogens with mucosal entry or colonization phases may improve overall vaccine efficacy.

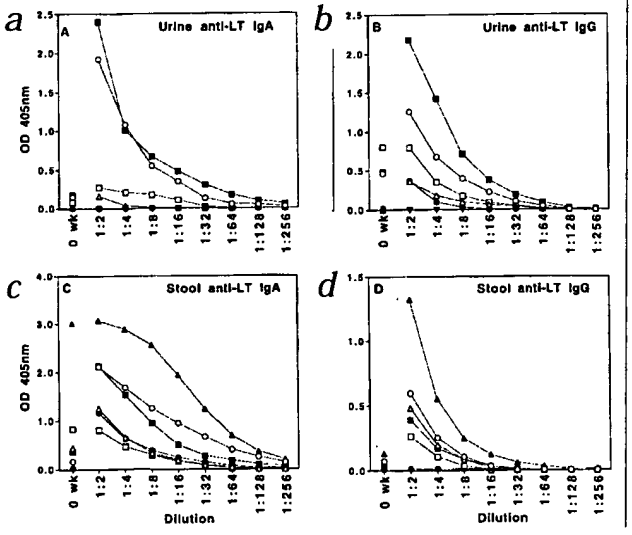

Fig. 2 Individual human mucosal antibodies in response to TCI. Volunteers were immunized transcutaneously with 500 μg of LT at 0, 12 and 35 weeks as described. Individual urine anti-LT IgA (*a*) and IgG (*b*), and stool anti-LT IgA (*c*) and stool anti-LT IgG (*d*) measured by ELISA as described. Data are shown as individual titration curves of specific anti-LT antibodies beginning at 1:2 dilution, and pre-immunization samples are shown in the first column with matching symbols for each individual.

Methods

Immunization. LT was produced under GMP conditions at the Swiss Serum and Vaccine Institute (Berne, Switzerland). Healthy volunteers with no history of atopy or other skin diseases were enrolled in the study. Volunteers received LT in 500 µl of saline at the doses described, absorbed on a 5 × 5 cm2 single-ply polyester-rayon gauze pad with polyethylene backing covered by a 10 × 12 cm^2 Tegaderm dressing (3M Health Care, St. Paul, Minnesota). The patch was placed on the upper arm for 6 h, after which it was removed and the site was rinsed with 500 ml of sterile saline. Individuals were re-immunized after 12 wk and individuals in the 500-µg group were immunized a third time at 35 weeks using 250 µg of LT in 500 µl of saline on each arm. Volunteers were seen after each immunization on days 1, 2, 3 and 7 and examined for signs of inflammation at the site of immunization.

Biopsy of immunization sites. A 4-mm punch biopsy was taken from the site of immunization and the contralateral arm of one volunteer at 24 h and a second volunteer at 48 h after the second immunization. At the time of the third immunization, one volunteer wore both an LT-loaded patch and a sham patch containing saline but no LT. A 4-mm punch biopsy was taken from both the site of immunization and at the site of the sham patch. Haematoxylin and eosin (H&E) staining and staining for CD1a was performed as described[13]. All procedures were performed after WRAIR institutional approval and volunteer consent.

Measurement of human anti-LT antibody titers. Serum, urine, and stool specimens were collected at the same time points (see Fig. 1) and antibodies against LT were detected as previously described with minor modifications[24,25]. The stool was homogenized, centrifuged and stored with protease inhibitor (5% dry milk, 0.2 µM AEBSF, 1.0 µg/ml aprotinin and 10.0 µM leupeptin in PBS) to prevent antibody degradation. Stool sample supernatant was stored at −20 °C. Serum IgA anti-body against human was determined using goat IgA(α)-HRP against human (Kirkegaard and Perry, Gaithersburg, Maryland), and quantitated (ng/ml) against a standard IgA curve (Sigma). Serum IgG antibody results are reported in ELISA units (EU), defined as the inverse dilution of sample that yields an OD of 1.0. Urine and stool antibody results are shown as titration curves using the most positive samples and are compared to pre-immunization samples. Total IgG and IgA were determined for stool and urine samples using human serum IgG and human IgA colustrum as standards (Sigma). The specific stool and urine IgG or IgA antibody was normalized (specific/total) against the total IgG or IgA in each sample and reported as positive if there was a greater than four-fold rise in the normalized antibody titer compared with the 0-wk specimen at more than three time points.

Although there are significant differences in the thickness and total lipid content of murine and human stratum corneum[8], TCI seems to allow passage of a large antigen such as LT (86 kD) through the stratum corneum into the epidermis in humans, as it does in mice. Although the mechanisms of stratum corneum penetration in TCI are not well understood[19], the importance of hydration for humans is reinforced by the observation that TCI using hydration is effective in other mammalian species[20].

Although volunteer biopsies were taken primarily to confirm the absence of microscopic inflammation, we concurrently observed morphological changes in epidermal LCs in response to LT applied to the skin. A role for LCs in the induction of systemic immune responses to TCI seems likely because LCs are the only antigen-presenting cells in the uninflamed epidermis[21]. The potent human immune responses induced by TCI did not require recruitment of inflammatory cells or result in clinically apparent reaction at the site of immunization. After extensive study, LCs have been confirmed to have a role in 'gene-gun' immunization using the epidermis[22], but the role of LCs in TCI remains to be determined.

TCI appears to be broadly applicable, enabling the use of a variety of adjuvants[23]. Factors such as antigen concentration, patch configuration and skin preparation may improve the efficiency of TCI in humans as it has in mice[5,23]. The present study indicates that immunization using an adjuvant in a patch on human skin is a simple, practical innovation that may improve the delivery of vaccines and enhance vaccine efficacy.

Acknowledgments

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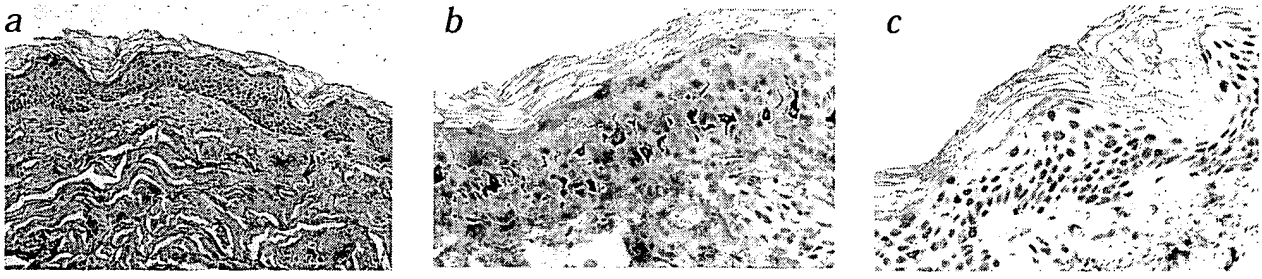

Fig. 3 Human skin sections from immunized volunteers. Haematoxylin and eosin (H&E) stain of biopsy from the site of immunization at 48 h (***a***), showing an absence of inflammatory infiltrate. H&E staining of biopsy in the same subject from the unimmunized arm was also normal. Langerhans cells stained using anti-CD1a from the same subject at site of immunization at 48 h (***b***) demonstrating changes to LC morphology and compared to normal findings on the opposite arm (***c***). Similar findings were seen using other human LC markers S-100 and HLA-DR. Magnification: ×200.

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Artificial antigen-presenting cells as a tool to exploit the immune 'synapse'

BERENT PRAKKEN[1], MARCA WAUBEN[2], DAVIDE GENINI[3], RODRIGO SAMODAL[1], JOELLEN BARNETT[1], ALBERTO MENDIVIL[1], LORENZO LEONI[3] & SALVATORE ALBANI[1,4].

[1]Department of Pediatrics, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0663, USA, [2]Department of Infectious Diseases & Immunology, Utrecht University, Faculty of Veterinary Medicine, PO Box 80165, 3508 TD Utrecht, The Netherlands
[3]Department of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0663, USA
[4]Androclus Therapeutics, Contrada Torre Allegra, 95030 Catania, Italy
Correspondence should be addressed to S.A., email: salbani@ucsd.edu

Recent progress in molecular medicine has provided important tools to identify antigen-specific T cells. In most cases, the approach is based on oligomeric combinations of recombinant major histocompatibility complex–peptide complexes fixed to various rigid supports available for binding by the T-cell receptor[1–8]. These tools have greatly increased our insight into mechanisms of immune responses mediated by CD8⁺ T cells[1,2]. Examples of the diverse fields of application for this technology include immunization, viral infections and oral tolerance induction[1–6].

A stable interaction between antigen-presenting cells (APC) and T cells is dependent not only on the absolute affinity between the T-cell receptor (TCR) and its ligand but also on the relative density of molecules available for contact at the interaction site[9]. The proper ligand density is achieved by migration of the relevant molecules toward the initial interaction site, a phenomenon called 'capping', whose outcome is the formation of the immune 'synapse', the machinery required for T-cell signaling[9]. At present, methods for the detection of antigen-specific T cells rely only on absolute affinity between the ligands and do not allow the physiologic phenomenon of capping. This may limit the use of these tools to detection, not manipulation, of antigen-specific T cells. Low-affinity interactions, which may be involved in processes such as autoimmunity, may also be missed[10–14]. So far, examples of successful detection of class II-restricted antigen-specific T cells by oligomeric major histocompatibility complex (MHC)–peptide complexes are also limited[1,2,15–17].

We have developed a system that mimics the physiological interactions among T cells and APC. We use artificial antigen-presenting cells (aAPC), composed of a liposome, in which MHC class II–peptide molecules are incorporated. The composi-